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Red Blood Cell Alloimmunization in Sickle Cell Disease: The Influence of Racial and Antigenic Pattern Differences Between Donors and Recipients in Brazil

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Red blood cell (RBC) transfusions are widely used in the management of patients with sickle cell disease (SCD). However, repeated RBC transfusions are often complicated by RBC alloimmunization. To investigate whether the frequency of RBC alloimmunization could be accounted for by racial and RBC phenotype differences between donors and recipients in Brazil, in this study we compared the RBC phenotype of 100 SCD patients with that observed in 120 randomly selected blood donors. A comparison of the RBC phenotype between the two groups revealed a statistically significant increase in the frequency of the C antigen in the donor population ($P < 0.01$), but no significant difference was observed for the A, B, D, c, E, e, K, k, Fy^a, M, N, S, s, and Jk^a antigens. Using standard techniques (indirect antiglobulin test, enzyme treatment, and low-ionic-strength solution) we observed an RBC alloimmunization rate of 12.9% (11/85) in the SCD patients. Fifteen alloantibodies were detected in 11 patients, and most (80%) involved antigens in the Rhesus and Kell systems. This observed RBC alloimmunization rate in SCD patients in Brazil is lower than that reported by studies from North America, suggesting that the requirement for extended antigen-matched RBC transfusion for SCD patients in the setting of a RBC phenotype concordant donor-recipient population may not be cost-effective in some countries. © 1996 Wiley-Liss, Inc.

Key words: sickle cell disease, alloimmunization, red-cell antigens, blood transfusion

INTRODUCTION

Red blood cell (RBC) transfusions are frequently used in sickle cell disease (SCD) patients to reverse or prevent life-threatening events, improving oxygen carrying capacity and microvascular blood flow by increasing the haematocrit level and decreasing the percentage of HbS [1]. Acute simple transfusions may be used to treat sequestration crisis, aplastic crisis, accelerated heamolysis, blood loss, and in preoperative preparation [2]. Chronic simple transfusion is recommended for individuals who have cerebrovascular disease, debilitating vaso-occlusive symptoms, cardiopulmonary disease, and complicated pregnancy [2]. Exchange transfusion is performed in cerebrovascular disease, priapism, hepatic failure, and preoperative preparation [2]. Nevertheless, repeated RBC transfusions are often complicated by RBC alloimmunization, which results in difficulty to obtain compatible blood, acute or delayed transfusion reactions, and hemolytic disease of the newborn.

The rate of RBC alloimmunization in SCD patients has been reported to be about 20% with a range in North America from 8 to 50% [3–6]. Age, sex, number and time of transfusions, genetic factors, and racial differences between SCD patients and blood donors are variables implicated in the formation of RBC alloantibodies [7–10].

Considering the large variation described in the literature of the RBC alloimmunization rate in the SCD patients, and the importance of racial and phenotype differences among SCD patients and blood donors in the development of this complication, in the present study we determined the RBC phenotype, transfusion history, and RBC alloimmunization rate of patients with SCD in

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Brazil. In addition, the RBC phenotype of SCD patients and randomly selected blood donors were compared.

MATERIALS AND METHODS

Study Population

The RBC phenotype and transfusion history of 100 Brazilian patients with SCD [42 male and 58 female; 89 HbSS, 8 HbSC and 3 HbSβ-Tal; median age = 10.5 years (6 months to 43 years)] were analysed in a cross-sectional study. Eighty-five patients had received at least one transfusion and 15 had never been transfused. In the transfused group, the total number of RBC units received was 1,300 (range 1–150, mean = 15.3 U).

To investigate whether the frequency of RBC alloimmunization could be accounted for by racial and RBC phenotype differences between blood donors and recipients, we compared the RBC phenotype and the racial background of the 100 SCD patients with those observed in 120 randomly selected blood donors. SCD patients and blood donors were racially classified in white, black, or mulatto, according to skin colour, lip thickness, and hair aspect [11].

Blood samples from SCD patients and blood donors were phenotyped, using standard techniques recommended by the manufacturers for the following red-cell antigens: A, B, D, C, c, E, e, K, k, M, N, S, s, Fy^a, and Jk^a [12]. The screening for the presence of RBC alloantibodies was performed according to standard methods including enzymatic (papain 0.1%), antiglobulin, low-ionic strength solution (LISS), and manual Polybrene® techniques [12]. If the RBC alloantibody screening was positive, the antibody was further identified by testing the serum against a panel of previously typed reagent RBCs.

Statistical Methods

The comparison of the RBC antigenic frequency between groups of SCD patients and blood donors were analysed using a Chi-Square test. In some cases, when Cochran restrictions were detected, the Fisher's test was used. The statistical significance level was chosen to be 0.05.

RESULTS

The racial classification of the group of patients with SCD was: white (20%), black (36%), and mulatto (44%); while the group of blood donors was formed by: white (45%), black (12%), and mulatto (43%). The comparison between the two groups showed a statistically significant increase in the frequency of non-white individuals among the SCD patient group ($P < 0.001$).

The RBC alloimmunization rate among the SCD patients was 12.9%. Fifteen RBC alloantibodies were detected in 11 of the 85 patients who had been previously transfused (four patients developed two alloantibodies). Eighty percent of the RBC alloantibodies had anti-Rh and anti-Kell specificities. Table I shows the specificity of the RBC alloantibodies found in patients with SCD. The risk of red-cell alloimmunization defined as the total number of alloantibodies divided by the total number of transfused RBC units × 100 was 1.15%. The alloimmunized patients (7 male and 4 female) received a mean of 19 RBC transfusions, while the group of non-alloimmunized individuals received a mean of 14 RBC transfusions. The majority of RBC alloantibodies were detected among the large group of SCD patients who had been transfused with less than 10 RBC units. However, no significant difference was observed between the rate of alloimmunization of SCD patients transfused with less or more than 10 RBC units ($P = 0.6$) (Table II).

TABLE I. Specificity of RBC Alloantibodies Detected in Patients With SCD in Brazil

RBC alloantibody specificity	Number of RBC alloantibodies
Anti-D	03
Anti-C	03
Anti-E	02
Anti-K	02
Anti-c	01
Anti-e	01
Anti-Fy	01
Anti-Le	01
Total	15

TABLE II. Number of RBC Alloantibodies Detected in Brazilian Patients With SCD According to the Number of Transfused RBC Units

Number of transfused RBC units	Number of transfused SCD patients	Number of RBC alloantibodies
0	15	0
1–10	56	10
11–20	9	1
21–30	3	0
31–40	1	0
41–50	6	3
50+	10	1
Total	100	15

The comparison of the RBC phenotypes between the group of patients with SCD and the group of blood donors revealed a statistically significant increase in the frequency of the C antigen in the donor population ($P < 0.01$), but no statistical significant difference was observed in the frequency of the other tested red-cell antigens (Table III).

DISCUSSION

Several studies have indicated that the prevalence of RBC alloimmunization in SCD patients is higher than

TABLE III. Comparison Between the Frequency of RBC Antigens in SCD Patients and Blood Donors in Brazil*

RBC antigen	Blood donors (%)	Patients with SCD (%)	*P* value
D	87.5	93.0	NS
C	66.7	46.0	<0.01
c	85.0	93.0	NS
E	33.3	39.0	NS
e	96.7	96.0	NS
K	7.5	7.0	NS
k	100	100	NS
Fy	53.3	47.0	NS
Jk	80.0	81.0	NS
M	80.0	74.0	NS
N	85.8	85.0	NS
S	36.7	33.0	NS
s	87.5	89.0	NS

*NS, not significant.

that observed in other groups of multitransfused patients [6,9,10]. In the present study, we observed an RBC alloimmunization rate of 12.9% in Brazilian SCD patients, and a calculated risk of alloimmunization per transfused RBC unit of 1.15%. These rates are similar, or even smaller, to that observed in other groups of multitransfused patients due to haematologic or non-haematologic diseases [13–15]. Eighty percent of the RBC alloantibodies found in the group of Brazilian patients with SCD showed anti-Rh and anti-Kell specificities, which is in concordance with the results reported by studies of RBC alloimmunization in SCD patients in North America [3,6].

The mechanisms underlying the increase incidence of alloimmunization in patients with SCD has been suggested to be due to an altered immune response, increase frequency of certain HLA antigens, and/or a lack of phenotypic compatibility between blood donor and blood recipient [16,17].

Some investigators have also related the increased occurrence of RBC alloimmunization to an increased number of RBC units transfused [18]. In this study, no significant difference was observed between the rate of alloimmunization of SCD patients transfused with less or more than 10 RBC units, indicating no correlation between alloimmunization rate and number of units of blood transfused. However, the present data may be insufficient to state definitively that age or numbers of units transfused do not affect the rate of RBC alloimmunization. That is because the median age of the patient population was only 10.5 years and few of these patients had received large numbers of RBC transfusions. The calculated risk of alloimmunization of 1.15% observed in our Brazilian patients with SCD, is quite similar to the calculated risk of red-cell alloimmunization of 1% per unit of blood transfused in the general population [19], suggesting that Brazilian SCD patients are not at a greater risk of RBC alloimmunization than the general population.

Although we observed a statistically significant higher prevalence of non-white individuals among SCD patients compared to the blood donor population, the red-cell phenotyping was quite similar in the two groups, revealing a high rate of racial admixture. These findings are in contrast to those previously related in the literature. In North America, the racial background and the RBC antigenic pattern of the donor blood population and SCD patients are considerably different, due to poor racial admixture and the fact that most blood donors are white. Therefore, African American blood recipients are at greater risk of RBC alloimmunization because they are frequently transfused with RBC units donated by Caucasian individuals [9,17].

Some investigators have suggested the use of intraracial transfusions and blood extended matched for other antigens than A, B, and Rh_0 for SCD patients, in order to reduce the alloimmunization risk [4,5,10,20]. In contrast, this strategy has been suggested to be not cost-effective by other studies [19,21,22]. Based on our results, the use of extended matched transfusions in Brazil should be restricted to patients at risk and to patients who developed one or more RBC alloantibodies, since matching for antigens other than ABO or Rh_0 might increase costs dramatically [21]. In this context, the provision of blood selected to match the patient's red-cell phenotype only after an initial alloimmunization, recognizes that the majority of transfused SCD patients never become alloimmunized, and that the patient who develops an alloantibody may be at greater risk of becoming heavily immunized with multiple alloantibodies [23]. Therefore, unless new clinical tests become available to determine early which SCD patients are at high risk to develop RBC alloantibodies (responder patients), this may be a reasonable approach for these patients.

Although we observed a statistically significant increase in the frequency of the C antigen in the donor population, suggesting that all Rh_0 SCD individuals should be transfused with C negative blood, this approach might be questionable, since the C antigen is weakly immunogenic, with a described antigenic relative potency about 50 times smaller than the potency of the Kell antigen [19]. Thus, the requirements for an extended antigen-matched RBC transfusion for SCD patients may not be cost-effective in countries exhibiting a RBC phenotype concordant donor-recipient population such as that described in this Brazilian study.

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